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Perfluorooctane sulfonic acid and organohalogen pollutants in liver of three freshwater fish species in Flanders (Belgium): relationships with biochemical and organismal effects

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Hepatic perfluorooctane sulfonic acid contamination in Flanders (Belgium) might affect serological endpoints in feral carp and eel.

Abstract

A perfluorooctane sulfonic acid (PFOS) assessment was conducted on gibel carp (*Carassius auratus gibelio*), carp (*Cyprinus carpio*), and eel (*Anguilla anguilla*) in Flanders (Belgium). The liver PFOS concentrations in fish from the Ieperlee canal (Boezinge, 250–9031 ng/g wet weight, respectively) and the Blokkersdijk pond (Antwerp, 633–1822 ng/g wet weight) were higher than at the Zuun basin (Sint-Pieters-Leeuw, 11.2–162 ng/g wet weight) and among the highest in feral fish worldwide. Eel from the Oude Maas pond (Dilsen-Stokkem) and Watersportbaan basin (Ghent) had PFOS concentrations ranging between 212 and 857 ng/g wet weight. The hepatic PFOS concentration was significantly and positively related with the serum alanine aminotransferase activity, and negatively with the serum protein content in eel and carp. The hepatic PFOS concentration in carp correlated significantly and negatively with the serum electrolyte concentrations whereas a significant positive relation was found with the hematocrit in eel. Although 13 organochlorine pesticides, 22 polychlorinated biphenyl (PCB) congeners and 7 polybrominated diphenyl ethers (PBDEs) were also measured in the liver tissue, only PCB 28, PCB 74, γ-hexachlorocyclohexane (γ-HCH) and hexachlorobenzene (HCB) were suggested to contribute to the observed serological alterations in eel.

Keywords: Perfluorooctane sulfonic acid; Aminotransferase; Protein; Electrolyte; Hematocrit

1. Introduction

Perfluorooctane sulfonic acid (PFOS) is a widely used chemical with applications as a wetting and foaming agent and as a precursor of surfactants and pesticides (Abe and Nagase, 1982). It was recently shown to be a widespread environmental contaminant in aquatic and terrestrial biota. Top predators were shown to generally have the highest tissue concentrations suggesting biomagnification of PFOS (Giesy and Kannan, 2001). PFOS concentrations in feral fish are scarcely

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documented. In lake whitefish eggs from Michigan waters (USA), PFOS concentrations up to 380 ng/g wet weight have been measured and the maximal muscle and liver tissue PFOS concentrations reported in the USA were 300 ng/g wet weight and 170 ng/g wet weight, respectively. Tissue PFOS concentrations comparable to the highest ever measured (5140 ng/g wet weight in mink liver; Kannan et al., 2002) were found recently in the liver from plaice (*Pleuronectes platessa*) captured in the Western Scheldt estuary (The Netherlands) where the maximal liver PFOS concentration measured was 7760 ng/g wet weight (Hoff et al., 2003a). Also in fish from Etobicoke creek (ON, Canada) elevated PFOS liver concentrations in common shiner (*Notropus cornutus*) have been recorded ranging from 2 to 72.9 μg/g wet weight after an accidental spill of fire retardant foam at a nearby airport (Moody et al., 2002).

The biochemical effects of PFOS exposure have mainly been studied in mammalian model species in which PFOS was shown to be an inducer of peroxisomal β-oxidation (Ikeda et al., 1987; Sohlenius et al., 1993) and a hypolipemic agent (Haughom and Spydevold, 1992; Lau et al., 2001; Seacat et al., 2003). PFOS can also increase membrane fluidity (Hu et al., 2000) and inhibit gap junction intracellular communication (Hu et al., 2002). Effects on carboxylesterase expression (Derbel et al., 1996) have also been demonstrated in addition to developmental effects (York et al., 2000). An increase in serum alanine aminotransferase (ALT) activity was demonstrated in rhesus monkeys and in rats (Goldenthal et al., 1978a,b; Seacat et al., 2003). Hoff et al. (2003b) suggested that PFOS interferes with homeostasis of DNA metabolism and that PFOS induces liver damage in carp, as assessed by the serum ALT activity, while peroxisomal β-oxidation was not shown to be induced.

While preliminary PFOS biomonitoring campaigns have been conducted for wood mice (*Apodemus sylvaticus*; Hoff et al., 2004) and plaice and bib (*Trisopterus luscus*) in an estuarine environment (Hoff et al., 2003a), information for freshwater fish species in this context is lacking. Therefore, the liver PFOS concentrations, the serum ALT activity, the serum protein content, the hematocrit value, serum electrolyte levels, condition and growth rate have been measured in feral gibel carp, carp and eel. This allowed us to characterize the PFOS pollution degree at a number of freshwater locations in Flanders (Belgium) for the first time and investigate the relation between these biological endpoints and the liver PFOS concentration.

Aside from PFOS, the liver concentrations of 13 organochlorine pesticides, 22 polychlorinated biphenyl congeners and 7 polybrominated diphenyl ethers were measured because laboratory controlled experiments suggest that these compounds can possibly affect the endpoints under investigation in this study. This has been demonstrated for hexachlorobenzene exposed rats and workers in which an ALT activity increase has been observed (Almeida et al., 1997; Queiroz et al., 1998). Also PCB 126 exposure has been shown to induce the plasma ALT activity in birds (Hoffman et al., 1996) and Aroclors 1254 and 1260 have been demonstrated to be associated with increased serum ALT activities in rats (Mayes et al., 1998). The measurement of these organohalogens provided us with information on their possible involvement in the modulation of endpoints suggested to be affected by PFOS in this study.

2. Materials and methods

2.1. Sampling

In September and October 2002, eels that were in their yellow, pre-migratory stage were captured in the Ieperlee canal (Boezinge), the ponds Oude Maas (Dilsen-Stokkem), Blokkersdijk (Antwerp) and the Watersportbaan (Ghent) and Zuun basins (Sint-Pieters-Leeuw) in Flanders (Belgium). Carps were captured in the Blokkersdijk pond and the Zuun basin. Gibel carps were also collected at the latter location and in the Ieperlee canal (Fig. 1). Fykes were set up 2 days before the fish were collected. The numbers of fish captured are given in Table 1. The captured fish were anaesthetized with ethyl 3-aminobenzoate, weighed and the fork length was determined. Scales were taken from behind the head along the longitudinal axis. Blood was taken with caudal puncture. Serum was prepared by centrifugation (4000 rpm, 5 min) and frozen in liquid nitrogen. The liver was dissected and also stored in liquid nitrogen. Le Cren's condition factors were calculated and the age of the carp and gibel carp was determined by counting the scale annuli. The fish growth rate was calculated as described by the Fraser–Lee method (Bagenal and Tesch, 1978).

2.2. Serum biochemical assays

The serum alanine aminotransferase activity was determined by the spectrophotometric method of Bergmeyer et al. (1986). The serum Cl^-, Na^+, Ca^{2+} and K^+ levels were determined with ion-selective electrodes on a 9180 electrolyte analyzer (AVL Scientific, Roswell, GA, USA). The serum protein content was determined with the Bio-Rad Protein Assay (Bio-Rad, Munich, Germany). For the determination of the hematocrit, the relative red blood cell volume was determined after centrifugation of heparinized blood in sealed capillaries (2000 rpm, 5 min). For all these measurements, values for duplicate analyses varied maximally 14%.

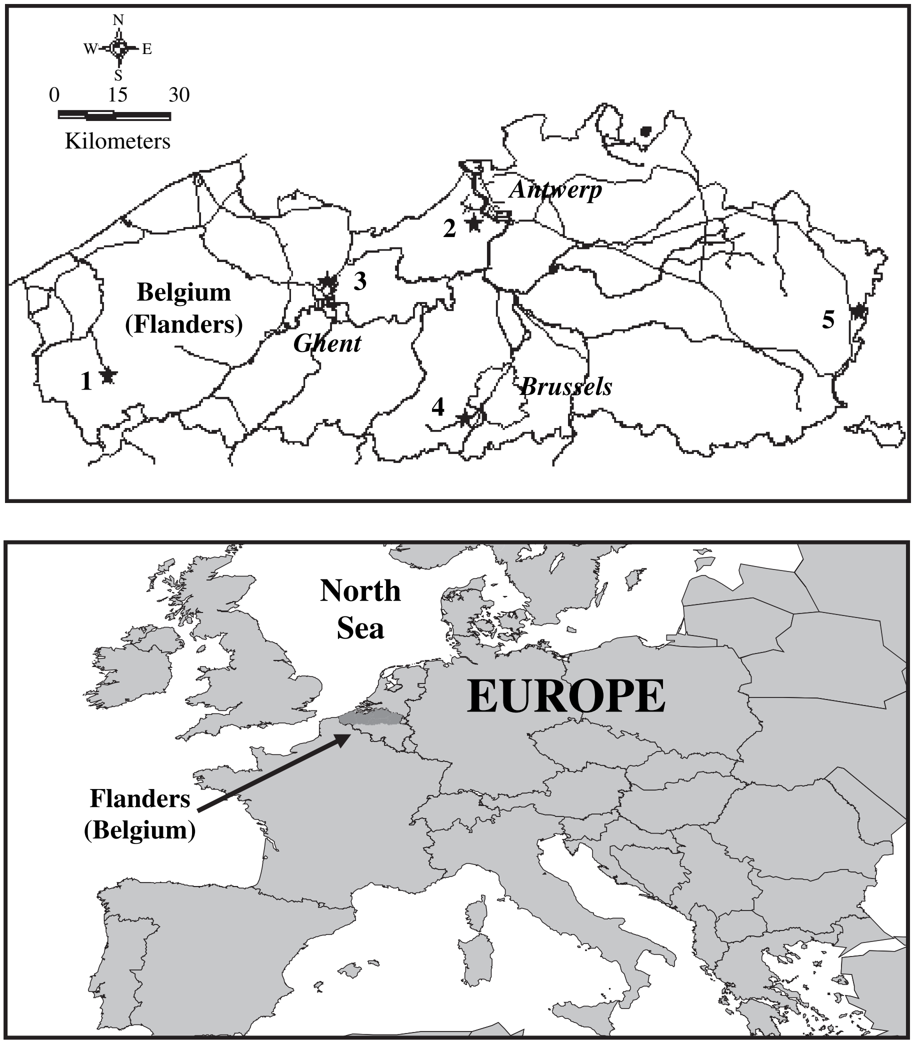

Fig. 1. Area of study and sampling locations. 1, Ieperlee canal; 2, Blokkersdijk pond; 3, Watersportbaan basin; 4, Zuun basin; 5, Oude Maas pond.

2.3. Determination of liver PFOS concentrations

The PFOS concentration in liver tissue (100–500 mg) was measured using combined high pressure liquid chromatography-mass spectrometry according to Giesy and Kannan (2001). High pressure liquid chromatography was done on a CapLC system (Waters, Milford, MA, USA) connected to a Quattro II triple quadrupole mass spectrometer (Micromass, Manchester, UK). Aliquots of 5 μl were loaded on an Optiguard C18 pre-column (10×1 mm inner diameter, Alltech, Deerfield, IL, USA). The analysis was performed on a Betasil C18 column (50×1 mm inner diameter, Keystone Scientific, San Jose, CA, USA) at a flow rate of 40 μl/min. The mobile phase was 2 mM NH_4OAc (A)/CH_3OH (B). A gradient elution was used starting at 45% B and going to 90% B in 3 min. After 5 min initial conditions were resumed. PFOS was measured under negative electrospray ionization using single reactant monitoring (m/z 499→99). The internal standard (1H, 1H, 2H, 2H-perfluorooctane sulfonic acid) was measured under the same conditions (m/z 427→81). The dwell time was 0.1 s. The electrospray-capillary voltage was set at −3.5 kV and the cone voltage was 24 V. The source temperature was 80 °C. The pressure in the collision cell was 3.3 10^{-5} mm Hg (Ar). The PFOS concentrations were determined with an unextracted calibration curve. Data quality assurance included laboratory blanks and continuing calibration verification. Precision and repeatability were 85%, and 74%, respectively.

2.4. Determination of liver concentrations of polychlorinated and polybrominated pollutants

The organochlorine pesticides under investigation were α-, β-, γ-isomers of hexachlorocyclohexane, p,p'-dichlorodiphenylethane (p,p'-DDE), p,p'-dichlorodiphenyldichloroethane (p,p'-DDD), o,p'-dichlorodiphenyltrichloroethane (o,p'-DDT) and p,p'-dichlorodi-

Table 1
Ranges and mean concentrations (in brackets) expressed in ng/g wet weight for organohalogens measured in liver of freshwater fish

Organohalogen	Gibel carp ($n=13$)	Carp ($n=12$)	Eel ($n=28$)
PFOS	11.2–781 (201)	11.3–1822 (934)	17.3–9031 (1387)
α-HCH	<LOD	<LOD–0.3	<LOD–0.3
β-HCH	<LOD	<LOD–0.2	<LOD
γ-HCH	<LOD–0.7	<LOD–0.6	0.2–2.3
p,p′-DDE	<LOD–2.5	4.3–56.0 (14.6)	1.8–122.7 (13.8)
p,p′-DDD	<LOD–2.0	3.7–14.0 (6.2)	0.6–26.0 (4.8)
p,p′-DDT	<LOD–0.4	<LOD–0.8	<LOD–4.8
o,p′-DDT	<LOD–0.9	<LOD–5.3	<LOD
QCB	<LOD	<LOD	<LOD
HCB	<LOD–0.8	<LOD–2.4	0.2–3.7 (1.3)
OxC	<LOD–0.6	<LOD–0.8	<LOD–5.3
TN	<LOD–2.8	<LOD–3.1	<LOD–5.5
TC	<LOD–1.8	<LOD–2.7	<LOD
CC	<LOD–1.1	<LOD–1.7	<LOD
PCB 28	<LOD–26.7	<LOD–6.8	<LOD–52.4
PCB 31	<LOD–16.3	<LOD–5.7	<LOD–14.2
PCB 74	<LOD–20.3	1.3–5.8 (3.4)	<LOD–19.8
PCB 95	<LOD–13.5	2.0–28.4 (11.9)	<LOD–18.0
PCB 99	<LOD–14.2	6.6–18.6 (11.9)	4.9–130 (47.4)
PCB 101	<LOD–12.8	11.3–23.4 (17.4)	2.2–40.0 (4.3)
PCB 105	<LOD–10.3	3.1–10.9 (5.0)	<LOD–33.1
PCB 110	<LOD–18.8	7.0–19.7 (14.8)	2.5–30.4 (11.2)
PCB 118	<LOD–29.0	11.2–23.2 (19.8)	6.8–81.9 (32.4)
PCB 128	<LOD–9.3	4.5–13.6 (7.4)	1.6–23.3 (5.5)
PCB 132	<LOD–7.5	3.2–9.1 (6.9)	<LOD–10.7
PCB 138	<LOD–34.5	15.3–69.5 (27.0)	7.0–104 (21.6)
PCB 149	<LOD–14.3	8.4–21.2 (16.6)	3.2–88.9 (31.4)
PCB 153	<LOD–53.8	22.4–126 (41.9)	14.7–257 (66.2)
PCB 156	<LOD–4.38	0.8–14.1 (3.2)	1.0–13.1 (2.2)
PCB 163	<LOD–11.7	4.0–21.5 (7.7)	0.8–29.5 (6.1)
PCB 170	<LOD–7.1	0.9–27.5 (6.4)	1.5–26.4 (4.5)
PCB 180	<LOD–18.1	3.8–62.3 (14.3)	3.0–91.7 (12.7)
PCB 183	<LOD–5.0	1.3–14.6 (3.5)	2.0–30.8 (6.2)
PCB 187	<LOD–9.4	<LOD–36.4	3.0–60.1 (11.9)
PCB 194	<LOD–0.9	<LOD–7.4	<LOD–9.7
PCB 199	<LOD–1.4	<LOD–7.9	<LOD–12.2
∑PCB	<LOD–323	110–568 (324)	83.1–690 (299)
PBDE 28	<LOD–0.2	<LOD–0.7	<LOD–0.2
PBDE 47	<LOD–10.3	0.3–4.6 (0.9)	0.6–33.1 (3.9)
PBDE 99	<LOD	<LOD–0.1	<LOD–0.4
PBDE 100	<LOD–0.9	<LOD–0.7	0.2–6.0 (1.1)
PBDE 153	<LOD–0.2	<LOD	<LOD–0.3
PBDE 154	<LOD–0.4	<LOD–1.2	<LOD–0.4
PBDE 183	<LOD	<LOD	<LOD
∑PBDE	<LOD–12.0	0.3–7.3 (1.0)	0.8–39.8 (5.2)

n, number of fish; LOD, limit of detection; PFOS, perfluorooctane sulfonic acid; HCH, hexachlorocyclohexane; DDE, dichlorodiphenylethane; DDD, dichlorodiphenyldichloroethane; DDT, dichlorodiphenyltrichloroethane; QCB, pentachlorobenzene; HCB, hexachlorobenzene; OxC, oxychlordane; TN, *trans*-nonachlor; TC, *trans*-chlordane; CC, *cis*-chlordane; PCB, polychlorinated biphenyl; PBDE, polybrominated diphenylether. ∑PCB and ∑PBDE were calculated by assuming that <LOD = 0 ng/g wet weight.

phenyltrichloroethane (*p,p′*-DDT), pentachlorobenzene (QCB), hexachlorobenzene, oxychlordane (OxC), *trans*-nonachlor (TN), *trans*-(TC) and *cis*-chlordane (CC). The following polychlorinated biphenyl congeners (International Union of Pure and Applied Chemistry numbers) were targeted: 28, 31, 74, 95, 99, 101, 105, 110, 118, 128, 132, 138, 149, 153, 156, 163, 170, 180, 183, 187, 194 and 199. Polybrominated diphenyl ether congeners 28, 47, 99, 100, 153, 154 and 183 were also included. The method used for sample preparation and analysis was described in detail by Jacobs et al. (2002); Voorspoels et al. (2003). Briefly, the available amount of tissue (50–500 mg) was ground with Na_2SO_4, internal standards were added and the mixture was extracted for 2 h with 75 ml hexane/acetone (3:1, volumetric ratio) into a hot Soxhlet manifold. After concentration, the extract was subjected to clean-up on acidified silica and analytes were eluted with 15 ml *n*-hexane followed by 10 ml dichloromethane. The eluate was concentrated to 80 μl and transferred to an injection vial. PCBs were determined on a HP 6890 gas chromatograph with electron capture detection (Hewlett Packard, Palo Alto, CA, USA) equipped with a 50 m × 0.22 mm × 0.25 μm HT-8 capillary column (SGE Scientific, Zulte, Belgium). PBDEs and organochlorine pesticides were determined on a HP 6890 gas chromatograph-5793 mass spectrometer (Hewlett Packard, Palo Alto, CA, USA) which was operated in negative chemical ionization and selected ion monitoring and was equipped with a 25 m × 0.22 mm × 0.25 μm HT-8 capillary column. Instrumental operating conditions and quality control were detailed presented by Jacobs et al. (2002); Voorspoels et al. (2003). Briefly, daily check of calibration curves, regular analysis of procedural blanks and of certified material CRM 350 (PCBs in mackerel oil) were included in the quality assurance protocol. Additionally, the method was tested by participation in several interlaboratory tests. Recoveries of target compounds ranged between 72 and 103%. Method limits of detection for individual PCB congeners ranged between 0.5 and 1 ng/g wet weight, while for organochlorine pesticides and PBDEs, they were 0.2 and 0.1 ng/g wet weight, respectively.

2.5. Statistical analysis

The non-parametric Mann–Whitney *U*-test was used for comparison of the gibel carp and carp hepatic PFOS concentrations and body weights at the different sampling locations. This test was also used to compare the gibel carp and eel hepatic PFOS concentrations and body weights from the Ieperlee canal. The eel liver PFOS concentrations and body weights at each location were compared with the non-parametric Kruskal–Wallis test with Dunn's test as post hoc criterion. The latter tests were also used to compare the liver PFOS concentrations and body weights of gibel carp, carp and eel from the Zuun basin.

The relationship between the measured pollutants and the biological endpoints was investigated with partial least squares analysis (PLS). The variables for which a numeric value was obtained in <30% of the observations and for which the variance was close to

zero were not used for PLS analysis. If PLS did not yield a relevant model, correlation analysis was used to study the relation between a selected set of organohalogens and biological endpoints. Correlation analysis was carried out for those compounds which were quantifiable in >50% of the individuals.

3. Results

The measured hepatic PFOS concentrations of the three fish species are shown in Fig. 2. The hepatic PFOS concentration in eels and gibel carps from the Ieperlee canal were significantly higher than in eels and gibel carps from the Zuun basin, respectively. Also the liver PFOS concentration in carps from Blokkersdijk was significantly elevated in comparison to carps from the Zuun basin. The liver PFOS concentrations in eels from the Zuun basin were found to be lower than at the Oude Maas pond. Eels from the Watersportbaan basin had lower hepatic PFOS concentrations than eels from the Ieperlee canal. Gibel carps from the Ieperlee canal and the Zuun basin had significantly lower liver PFOS concentrations than eels from these respective locations. The hepatic PFOS concentration of carps and gibel carps from the Zuun basin did not differ significantly, neither did the hepatic PFOS concentration of carps and eels from the Zuun basin.

The gibel carp body weight at the Ieperlee canal was significantly higher than at the Zuun basin while the body weight for carp at the latter location was significantly higher than at the Blokkersdijk pond. The eel body weight did not differ significantly among locations. Gibel carps from the Ieperlee canal and the Zuun basin had significantly higher body weights than eels from these respective locations. The body weight of carps and gibel carps from the Zuun basin did not differ significantly, neither did the body weight of carps and eels from the Zuun basin.

Table 1 gives an overview of the hepatic organohalogen concentrations measured. The PLS models describing the relations between the measured pollutant levels and the assessed biological endpoints, showed poor relationships for eel, carp and gibel carp ($Q^2 = 0.11$, $Q^2 = 0.10$, $Q^2 = 0.15$, respectively).

Table 2 shows the relationships between the liver PFOS concentration and the biological endpoints. In carp, the liver PFOS concentration was positively and significantly related to the serum ALT activity (expressed in U/g protein), and significant negative relations were found with the serum protein concentration and the serum Cl^-, Na^+ and Ca^{2+} concentrations. In eel, significant positive relationships were observed between the hepatic PFOS concentration and the serum ALT activity (expressed in U/g protein and U/l) and the hematocrit and a significant negative relationship was

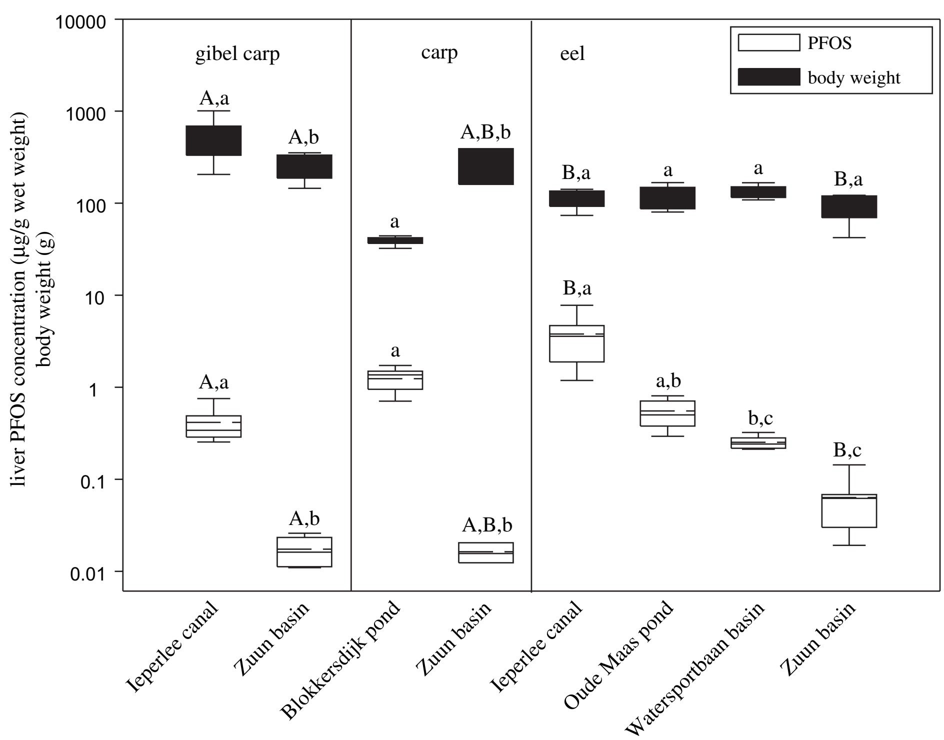

Fig. 2. Liver PFOS concentrations and body weight of gibel carps, carps and eels at each sampling location. The straight line is the median and the dotted line represents the mean. The 25th and 75th percentiles define the boxes. The whiskers represent the 10th and 90th percentiles. The lower case a, b and c indicate significant differences within species. The capitals A and B indicate significant differences between species for identical sampling locations. Boxes having different letters are significantly different ($p < 0.05$). PFOS, perfluorooctane sulfonic acid.

Table 2
Correlation analysis of the relationship between the hepatic PFOS concentration and the biological endpoints investigated

Correlation: liver PFOS concentration versus	Gibel carp (n=13)	Carp (n=12)	Eel (n=28)
Serum ALT activity (U/g protein)	r=0.16, p=0.62	r=0.70, p=0.014*	r=0.64, p=0.0003***
Serum ALT activity (U/l)	r=0.18, p=0.58	r=0.03, p=0.93	r=0.63, p=0.0007***
Serum protein content	r=−0.035, p=0.92	r=−0.74, p=0.0078**	r=−0.41, p=0.029*
Hematocrit	r=−0.50, p=0.081	r=−0.21, p=0.56	r=0.53, p=0.020*
Serum Cl^- concentration	r=−0.36, p=0.25	r=−0.73, p=0.013*	r=−0.34, p=0.068
Serum Na^+ concentration	r=−0.41, p=0.18	r=−0.79, p=0.0033**	r=−0.31, p=0.13
Serum Ca^{2+} concentration	r=−0.007, p=0.99	r=−0.86, p=0.0005***	r=−0.026, p=0.90
Serum K^+ concentration	r=0.50, p=0.10	r=−0.33, p=0.30	r=0.39, p=0.055
Le Cren's condition factor	r=−0.46, p=0.11	r=−0.49, p=0.13	r=0.0059, p=0.98
Growth rate	r=−0.083, p=0.84	r=0.59, p=0.061	ND

PFOS, perfluorooctane sulfonic acid; ALT, alanine aminotransferase; ND, not determined; r, correlation coefficient p, p value; n, number of fish. Spearman's rank correlation, *p<0.05, **p<0.01, ***p<0.001.

observed with the serum protein concentration. Graphical representations of these relationships are shown in Figs. 3 and 4.

Table 3 shows the relations between the PCB 28, PCB 74, γ-HCH and HCB concentrations and the biological endpoints shown to correlate significantly to the liver PFOS concentration in eels. This analysis shows that latter organohalogens are significantly related to these biological endpoints. For carp, none of the measured organohalogens aside from PFOS correlated significantly with the endpoints that correlated significantly with PFOS.

4. Discussion

The measurement of the liver PFOS levels showed that the concentrations in gibel carp and eel captured in the Ieperlee canal at Boezinge ranged from 250 to 781 ng/g wet weight and from 1024 to 9031 ng/g wet weight, respectively. Carp from the Blokkersdijk pond (Antwerp) had liver PFOS concentrations ranging between 633 and 1822 ng/g wet weight, an observation that is in good accordance with the high liver PFOS concentrations (mean and median values of 26.18 and 5.06 μg/g wet weight, respectively) measured in wood mice from Blokkersdijk (Hoff et al., 2004). The PFOS concentrations in the liver tissue of fish from the Ieperlee canal and the Blokkersdijk pond are higher than the highest PFOS liver concentration measured in fish liver tissue in the USA (170 ng/g wet weight in Chinook salmon liver captured in the Great Lakes, Giesy and Kannan, 2001) and are comparable with the highest liver PFOS concentration measured in wildlife to date (5140 ng/g wet weight in mink liver, Kannan et al., 2002). The elevated liver PFOS concentrations in the Ieperlee fish show that high tissue PFOS concentrations are not only found in the proximity of fluorochemical production units as is the case for the nature reserve Blokkersdijk, but might also occur in industrialized areas with no apparent perfluorochemical production activity. This is clearly the case for the Ieperlee canal at Boezinge since this sampling location is located downstream of the industrial zone of the city of Ypres suggesting PFOS (or its precursors) release into the Ieperlee canal via industrial and/or household wastewater discharges.

The observed differences in hepatic PFOS concentration between fish species at the same sampling location and within species for different sampling locations could be explained by differences in PFOS concentrations in water and sediment within and between locations, differences in PFOS tissue dilution extent, species-specific differences in nutritional habits, uptake/depuration and differences in ecological characteristics. Eel is an epibenthic species, for example, while carp and gibel carp are pelagic species. Also the nutritional habits of the investigated species differ: eel is carnivorous and carp and gibel carp are omnivorous species (Vandelannoote et al., 1998). It is currently unknown, however, how these factors could affect PFOS accumulation in liver tissue.

The strongly significant correlations between the liver PFOS concentration and the serum ALT activity in eel and carp observed in this study suggest that PFOS may induce hepatic damage in these species in the field. In an earlier report, PFOS has been demonstrated to be significantly related to the serum ALT activity in juvenile bibs from the Western Scheldt although the relation was weak (r=0.44, p<0.05, Hoff et al., 2003a). The relations found in the present study are possibly stronger because of the larger liver PFOS concentration ranges (11.3–1822, 17.3–9031 ng PFOS/g wet weight in carp and eel, respectively) compared to bibs for which the liver PFOS concentrations ranged between 11 and 217 ng PFOS/g wet weight. Although the liver hepatic concentrations in plaice from the North Sea and the Western Scheldt ranged between 107 and 7760 ng/g wet weight, which is similar to the PFOS concentration range in eel measured in this study, no significant relation was found between the hepatic PFOS concentration

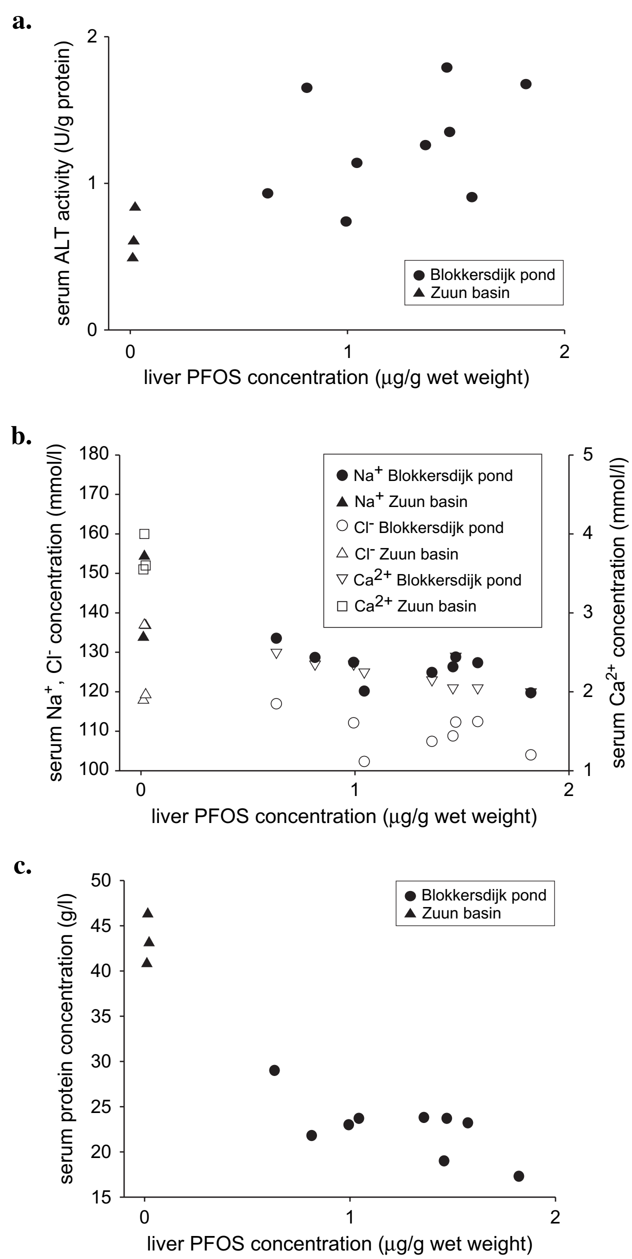

Fig. 3. The relationships between the carp liver PFOS concentration and the serum ALT activity (a), electrolyte concentrations (b) and serum protein concentration (c). PFOS, perfluorooctane sulfonic acid; ALT, alanine aminotransferase.

and the serum ALT activity in plaice which could be due to differences in species sensitivity. The positive significant relationship between the liver PFOS concentration and the serum ALT activity in eel and carp is consistent with a PFOS-mediated serum ALT activity increase observed in a short term exposure experiment in carp (Hoff et al., 2003b). It should be noted that the lowest observed effect concentration for serum ALT activity increase in the latter experiment was 561 ng PFOS/g wet weight. Comparison between this tissue value and the liver PFOS concentrations measured in this study, however, is difficult because intraperitoneal injection was used. An increase of serum ALT activity after PFOS exposure was also shown in a subacute rhesus monkey and two rat studies (Goldenthal et al., 1978a,b; Seacat et al., 2003). Seacat et al. (2003) showed that the mean liver PFOS no observed adverse effect level for male and female rats for an increase in serum ALT activity was 364 µg PFOS/g wet weight after 14 weeks of PFOS exposure. In the present study, the hepatic PFOS concentration was found to be significantly correlated to the serum ALT activity for PFOS concentration ranges well below the rat no observed effect level (11.3–1822, 17.3–9031 ng PFOS/g wet weight in carp and eel, respectively). Differences in species sensitivity or route of exposure could account for this observation. It should also be noted that the duration of exposure of the carps could be much longer than the experimental rat exposure as the age range for carps from the Blokkersdijk pond was 1.9–3.6 years. Also for the yellow eels captured in this study the exposure period might have been relatively long as feral eels can remain in the yellow pre-migratory stage for 7–20 years (Langston et al., 2002).

Other toxicants may have contributed to the alteration in serum ALT activity in eel and carp. In eel, the possibility that other organohalogenated contaminants but PFOS might have affected the serum ALT activity, the total protein concentration and the hematocrit is supported by the significant correlations found between the liver concentrations of PCB 28, PCB 74, γ-HCH, HCB and these endpoints. It is possible that these compounds could have contributed to the induction of the serum ALT activity because earlier studies report increased plasma ALT activities observed in HCB exposed rats (Almeida et al., 1997) and increased serum ALT activities in HCB exposed workers (Queiroz et al., 1998). Also PCB congeners could affect the ALT activity. PCB 26, for example, can induce the plasma ALT activity in birds (Hoffman et al., 1996) and Aroclors 1254 and 1260 have been demonstrated to be associated with increased serum ALT activities in rats (Mayes et al., 1998).

The significant relations between the hepatic PFOS concentration and the serum Cl^-, Na^+ and Ca^{2+} concentrations in carp suggest that PFOS could induce ion regulatory distress by disrupting membrane structure and/or function of gill cells which play a key role in osmoregulation and regulation of ion homeostasis (Wendelaar Bonga and Lock, 1992). Although not investigated under laboratory conditions, PFOS has been shown to have several effects on the membrane level: increasing of proton leakage of the inner mitochondrial membrane (Starkov and Wallace, 2002), enhancing of membrane fluidity in a rat liver hepatoma cell line and carp and chicken red blood cells (Hu et al., 2000) and inhibition of gap junction communication

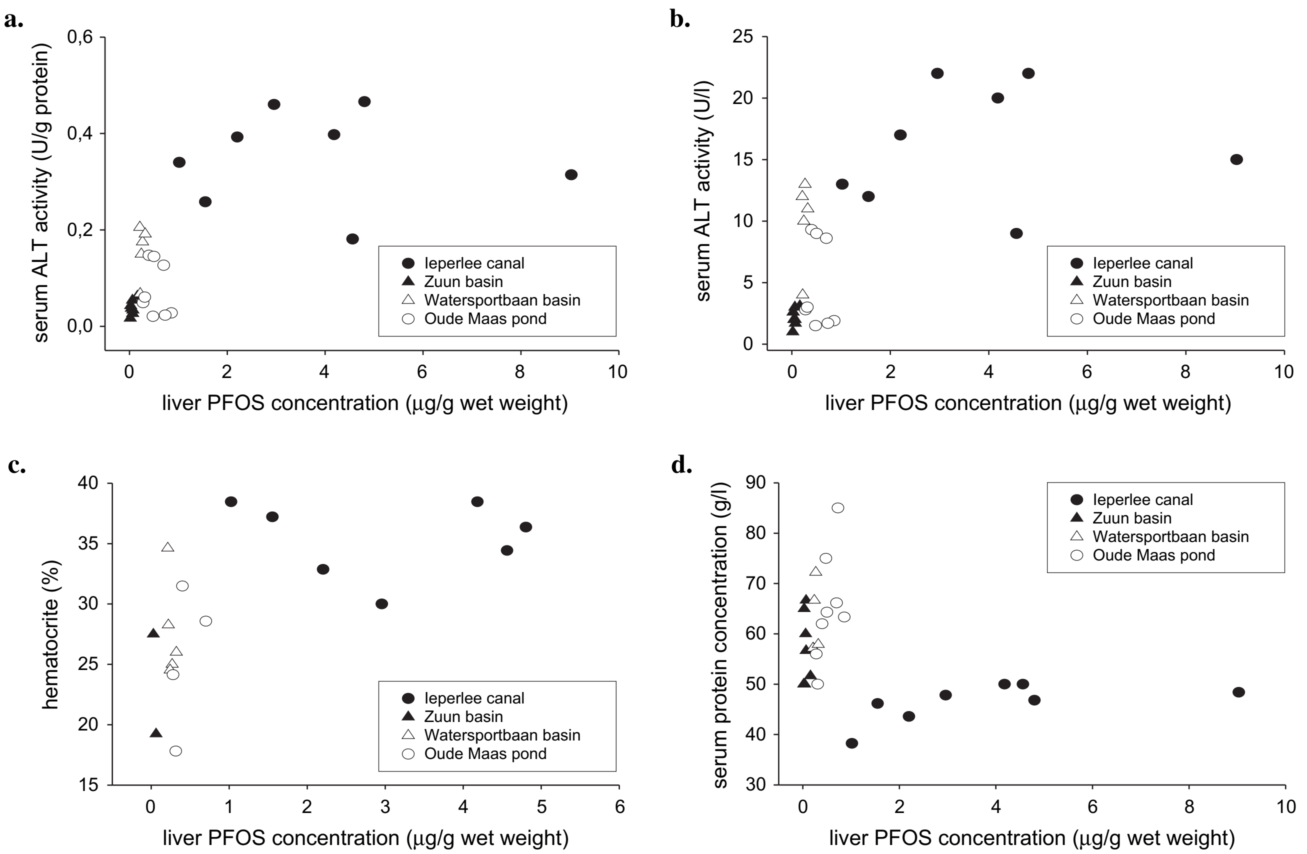

Fig. 4. The relationships between the eel liver PFOS concentration and the serum ALT activity (a,b), hematocrit (c) and serum protein concentration (d). PFOS, perfluorooctane sulfonic acid; ALT, alanine aminotransferase.

between cells (Hu et al., 2002). PFOS has an amphipatic structure and could physically disturb the structure of membranes as is the case for many detergent-like compounds (Schreier et al., 2000). Moreover, PFOS has been shown to induce hepatocyte damage in vivo (Hoff et al., 2003b) which might be indicative for a more general cytotoxic capacity, that of the gills included.

PFOS-mediated disturbance of gill structure in carp, suggested by the decrease in serum Cl^-, Na^+ and Ca^{2+} concentrations, can lead to hemodilution due to an increase in osmotic water uptake (Wendelaar Bonga and Lock, 1992). This could be an explanation for the observed decrease in serum total protein content associated with PFOS exposure as serum proteins might have been diluted in increased serum volumes. Hemodilution could also explain the lack of any significant relation between the hepatic PFOS concentration in carp and the serum ALT activity, expressed in U/l serum; an increase in serum volume due to hemodilution, could conceal an increase in ALT activity. This increase in ALT activity is suggested by the significant positive relation with the hepatic PFOS concentration when the ALT activity is expressed in U/g protein. It is also possible, however, that the latter relation indicates a decrease in total serum protein content and an unaltered ALT activity.

Table 3
Correlation analysis of the relationship between the hepatic PCB 28, PCB 74, γ-HCH and HCB concentrations and the biological endpoints investigated in eel

	Serum ALT activity (U/g protein)	Serum ALT activity (U/l)	Hematocrit	Serum protein content
PCB 28 ($n=22$)	$r=0.80$, $p<0.0001$***	$r=0.77$, $p<0.0001$***	$r=0.80$, $p<0.0001$***	$r=-0.45$, $p=0.040$*
PCB 74 ($n=21$)	$r=0.76$, $p<0.0001$***	$r=0.77$, $p<0.0001$***	$r=0.65$, $p=0.0066$**	$r=-0.47$, $p=0.035$*
γ-HCH ($n=28$)	$r=0.63$, $p=0.0005$***	$r=0.64$, $p=0.0002$***	$r=0.67$, $p=0.0016$**	$r=-0.48$, $p=0.0099$**
HCB ($n=28$)	$r=0.72$, $p<0.0001$***	$r=0.65$, $p<0.0001$***	$r=0.64$, $p=0.0029$**	$r=-0.65$, $p=0.0002$***

ALT, alanine aminotransferase; PCB, polychlorinated biphenyl; HCH, hexachlorocyclohexane; HCB, hexachlorobenzene; r, correlation coefficient; p, p value; n, number of fish. Spearman's rank correlation, *$p<0.05$, **$p<0.01$,***$p<0.001$.

At present it is difficult to explain the significant negative correlations between the hepatic PFOS concentration and the total serum protein content observed in carp and eel. It has been shown that PFOS binds to serum proteins and mainly to serum albumin (Jones et al., 2003) but the relation between total protein serum levels and hepatic PFOS concentrations has not been studied under controlled conditions.

In carp, the serum endpoints that correlated significantly with the liver PFOS concentration, did not correlate significantly with the other measured organohalogens. This could imply that the latter compounds are relatively unimportant determinants in the prediction of the serum ALT activity, the serum Cl^{-}, Na^{+} and Ca^{2+} concentrations and the serum protein concentration which were shown to be significantly associated with the liver PFOS concentration.

In contradiction to carps, the lack of changes in serum electrolyte concentrations in eels suggests that PFOS did not induce gill dysfunction in that species. Therefore, the mechanism underlying the observed serum protein concentration decrease in eels is most probably not due to hemodilution caused by gill dysfunction. The probable lack of hemodilution in eels could also account for the observation that the correlation between the liver PFOS concentration and the serum ALT activity, expressed relative to the serum volume, is significant in eels but not in carps.

The PFOS-associated hematocrit increase in eels, which was found to be associated with increased liver PFOS concentrations, might reflect swelling of erythrocytes, increase of erythrocyte numbers or dehydration. It would be speculative, however, to elaborate on the likelihood of any of these hypotheses.

Although some biological endpoints were suggested to be altered by PFOS exposure in this study, no indications were obtained for a decrease in fish condition or a reduced growth capacity mediated by PFOS exposure.

The different correlation patterns observed between fish species could suggest differences in PFOS toxicity mechanisms between species. The present study, however, does not allow to draw sound conclusions concerning this issue as capturing locations and PFOS liver concentrations were not similar for all the fish species.

5. Conclusions

In conclusion this study shows that the Blokkersdijk pond (Antwerp), situated in a nature reserve neighboring a fluorochemical production unit, and the Ieperlee canal at Boezinge (Ypres), a location downstream of an industrial area without apparent perfluorochemical production activity, are hot spots for freshwater fish PFOS pollution in Flanders (Belgium). At these locations the hepatic PFOS concentrations are among the highest concentrations ever reported for wildlife liver tissue. In eel and carp, the liver PFOS concentration correlated significantly and positively with the serum ALT activity, a marker for hepatic damage, showing that PFOS might induce liver damage in freshwater fish under field conditions. A decrease of the total serum protein content in carp and eel, disturbance of ion homeostasis in carp suggesting gill damage, and an increase of hematocrit levels in eel were also suggested to be PFOS-mediated although other measured organohalogens might be (partly) responsible for the observed biochemical alterations in eel. The hepatic PFOS levels were not shown to be significantly linked to fish growth or condition.

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